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# 植物在缺钾胁迫中膜稳定性的变化*

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**摘要**: 植物维持膜的功能是其抵御胁迫的关键问题, 而维持膜功能必须要保持膜的稳定性和合适的流动性。我们前期的研究发现植物主要是通过积累叶片膜脂和保持根部膜脂基本不变来适应长期缺钾。在本研究中, 以拟南芥和其具有耐受缺钾胁迫特性的近缘种须弥芥为对象, 研究了与膜的流动性密切相关的双键指数 (double bond index, DBI) 的变化, 发现长期缺钾条件下, 两种植物叶片中总的 DBI 保持不变, 根部总的 DBI 略有降低。同时研究了与膜稳定性密切相关的溶血磷脂的含量和 DGDG/MGDG 以及 PC/PE 这两个比值的变化, 发现长期缺钾后拟南芥和须弥芥叶片中溶血磷脂的总量呈上升趋势, 根部溶血磷脂总量基本保持不变; 无论在对照还是缺钾条件下, 拟南芥溶血磷脂的总含量要高于须弥芥。须弥芥叶片具有更高的 DGDG/MGDG 值, 根部具有更高的 PC/PE 值, 说明长期缺钾条件下须弥芥膜的稳定性可能更好。这可能是须弥芥耐缺钾的原因之一。

**关键词**: 须弥芥; 拟南芥; 耐缺钾; 膜流动性; 膜稳定性
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# Changes of Membrane Stability in Potassium-Stressed Plants

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**Abstract**: The maintenance of membrane function is critical to the ability of plants to resist environmental stresses; specifically, the stability and appropriate fluidity of membranes are crucial to their normal function. We previously demonstrated that plants adapt to long-term potassium ($K^+$) deficiency by accumulation of membrane lipids in leaves and maintenance of the lipid composition in roots. In this study, which involved *Arabidopsis thaliana* and its $K^+$-deficiency-tolerant relative *Crucihimalaya himalaica*, we first calculated the double-bond index (DBI) as an indicator of membrane fluidity. After exposure to long-term $K^+$-deficiency stress, the DBI of the total lipids in leaves of *A. thaliana* and *C. himalaica* showed no significant changes, whereas the DBI of the total lipids in the roots of these species showed slight increases. Changes in lysophospholipids (lysoPLs) levels, and digalactosyldiacylglycerol/monogalactosyldiacylglycerol (DGDG/MGDG) and phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratios, all of which strongly reflect membrane stability, were also studied in $K^+$-stressed *A. thaliana* and *C. himalaica*. After long-term $K^+$ deficiency, total lysoPLs levels increased in *A. thaliana* and *C. himalaica* leaves, but showed no significant changes in roots. DGDG/MGDG and PC/PE ratios were higher in *C. himalaica* leaves and roots than in those of *A. thaliana*. These results indicate that *C. himalaica* exhibits superior membrane stability compared with *A. thaliana*. This may explain its superior growth and tolerance under $K^+$-deficient conditions.

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**Key words**: *Crucihimalaya himalaica*; *Arabidopsis thaliana*; Tolerance of $K^+$ deficiency; Membrane fluidity; Membrane stability

**Abbreviations**: digalactosyldiacylglycerol, DGDG; monogalactosyldiacylglycerol, MGDG; phosphatidylglycerol, PG; phosphatidylcholine, PC; phosphatidylethanolamine, PE; phosphatidylinositol, PI; phosphatidylserine, PS; phosphatidic acid, PA; double-bond index, DBI; lysophospholipid, lysoPL; lysophosphatidylglycerol, lysoPG; lysophosphatidylcholine, lysoPC; lysophosphatidylethanolamine, lysoPE; electrospray ionization tandem mass spectrometry, ESI-MS/MS

In our previous study, we found that *Crucihimalaya himalaica*, a relative of *Arabidopsis thaliana*, is tolerant to $K^+$-deficient conditions (Wang *et al.*, 2014). Compared with *A. thaliana*, the higher ratios of root/shoot dry weight and shoot $K^+$/root $K^+$ in *C. himalaica* might account for its superior growth and lower levels of chlorosis under $K^+$-deficient conditions. Detailed analysis of the lipid composition in *A. thaliana* and *C. himalaica* indicated that plants adapt to long-term $K^+$ deficiency by the accumulation of membrane lipids in leaves and the maintenance of the lipid composition in roots (Wang *et al.*, 2014). In addition to adjustment of the lipid composition, the double-bond index (DBI) and level of lysophospholipids (lysoPLs) in plants also change under adverse conditions. It is well known that the degree of unsaturation of glycerolipids, which is reflected by DBI, affects membrane fluidity. DBI is the average number of double bonds in the two fatty acid chains of a glycerolipid molecular species. DBI is increased or decreased in order to enhance or reduce membrane fluidity as an adaptation to low-temperature or high-temperature stress, respectively.

LysoPLs, which include lysophosphatidylglycerol (lysoPG), lysophosphatidylcholine (lysoPC) and lysophosphatidylethanolamine (lysoPE), are minor constituents of membrane lipids, but the levels of lysoPLs are very sensitive to environmental stimuli. Previous studies indicated that mechanical damage (Lee *et al.*, 1997), low-temperature stress (Li *et al.*, 2008; Welti *et al.*, 2002; Zhang *et al.*, 2013) and abscisic acid-promoted senescence (Jia and Li, 2013) induced the accumulation of lysoPLs in plants. An increase in lysoPLs was also observed in drought- and salt-stressed *A. thaliana* and *Thellungiella halophila* plants (our unpublished data).

Different lipids have different effects on the properties of membranes. It has been reported that digalactosyldiacylglycerol (DGDG) and phosphatidylcholine (PC) are bilayer-preferring lipids; in contrast, monogalactosyldiacylglycerol (MGDG) and phosphatidylethanolamine (PE) tend to form a hexagonal II phase (Cullis *et al.*, 1986; Webb and Green, 1991), which has a strong propensity to result in membrane leakage. Many studies have shown positive correlations between stress tolerance and higher ratios of DGDG/MGDG and PC/PE (Chen *et al.*, 2006; Quartacci *et al.*, 1997; Suss and Yordanov, 1986; Toumi *et al.*, 2008; Zhang *et al.*, 2012). However, it remains unclear how DBI, lysoPLs, and the ratios of DGDG/MGDG and PC/PE change with long-term $K^+$ deficiency.

In this study, electrospray ionization tandem mass spectrometry (ESI-MS/MS) was employed to determine the changes in the levels of lysoPLs in $K^+$-stressed *A. thaliana* and *C. himalaica*. DBI was also used as an indicator of membrane fluidity. DGDG/MGDG and PC/PE ratios were calculated to estimate the membrane stability of shoots and roots, respectively. The results obtained indicate that, under $K^+$-deficient conditions, changes of the DBI of total lipids in the leaves of *A. thaliana* and *C. himalaica* differed from those of roots. The extent of the change in the levels of lysoPLs in $K^+$-stressed *A. thaliana* and *C. himalaica* leaves was larger than that in roots. In addition, the ratios of DGDG/MGDG and PC/PE were greater in *C. himalaica* than in *A. thaliana* under both control and $K^+$-deficient conditions.

## 1 Methods and materials

### 1.1 Plant material

Seed-germinated plantlets of *A. thaliana* (Columbia ecotype) and *C. himalaica* were used throughout this study. Seeds of *C. himalaica* were collected from the alpine cold desert soil of Baima Snow Mountain, which is in De Qin, Yunnan.

### 1.2 Growth conditions and $K^+$-deficient treatment

Seeds of *A. thaliana* and *C. himalaica* were planted on Murashige and Skoog medium and germinated in a growth chamber at 22 ℃ with 12 h light/12 h darkness photoperiod with a light intensity of 120 μmol $m^{-2}s^{-1}$. For further growth, plantlets were transferred to a hydroponic system (Tocquin *et al.*, 2003). To assess the effects of $K^+$-deficiency, *A. thaliana* and *C. himalaica* plants with rosettes of the same diameter (30-day-old *A. thaliana* plants and 35-day-old *C. himalaica* plants) were transferred to a modified hydroponic system with different potassium levels: $K^+$ sufficiency (5.1 mmol · $L^{-1}$ $KNO_3$), mild $K^+$ limitation (0.51 mmol · $L^{-1}$ $KNO_3$), severe $K^+$ limitation (0.051 mmol · $L^{-1}$ $KNO_3$), and $K^+$ deprivation (0 mmol · $L^{-1}$ $KNO_3$). The nitrogen level was balanced using $NH_4NO_3$ and the growth medium was changed every six days.

### 1.3 Lipid extraction and ESI-MS/MS analysis

Lipid extraction, ESI-MS/MS analysis, and lipid quantification were performed as described previously with minor changes (Welti *et al.*, 2002). Leaves and roots of the plants were collected separately and immediately transferred to isopropanol with 0.1% butylated hydroxytoluene at 75 ℃ to inhibit lipolytic activities. They were then extracted several times using chloroform/methanol (2∶1) with 0.1% butylated hydroxytoluene. The extracts were gathered and, when the extract solvent appeared white, the extraction procedure was terminated.

### 1.4 Data analysis

The Q-test was performed to remove discordant data. The remaining data were then subjected to one-way factorial ANOVA using SPSS 16.0. Differences between means were tested by Fisher's least significant difference (LSD) method. Double-bond index (DBI) was calculated using the following formula: DBI = [∑($N$ × mol% lipid)]/100, where $N$ is the total number of double bonds in the two fatty acid chains of each glycerolipid molecule (Zheng *et al.*, 2011).

## 2 Results and discussion

### 2.1 The patterns of DBI change differed between plastidic and extraplastidic lipids in *A. thaliana* leaves

In *A. thaliana* leaves, the DBI of DGDG and MGDG were higher than those of any other lipid class, whereas the DBI of PI and PS were the lowest (Table 1). The DBI of MGDG was 5.88, whereas that of PS was only 2.74. Under $K^+$-deficient conditions, changes of DBI of total lipids showed no significant difference compared with the control. Under $K^+$-deficient conditions, DBI changes in most lipid classes showed no significant difference compared with the control. The largest change in DBI occurred for PA, which showed a 6.84% increase under 0 mmol · $L^{-1}$ $K^+$ condition. Under $K^+$-deficient conditions, patterns of DBI change differed between plastidic and extraplastidic lipids. After long-term $K^+$ deficiency, the DBI of plastidic lipids showed a decrease, but that of extraplastidic lipids increased (Table 1). The extent of DBI change of extraplastidic lipids was larger than that of plastidic lipids. For example, the DBI of DGDG decreased 1.32%, 1.32% and 0.38% under conditions of 0.51, 0.051 and 0 mmol · $L^{-1}$ $K^+$, respectively, compared with the control. In contrast, the DBI of PC showed increases of 2.30%, 3.57% and 3.57% under the same $K^+$ conditions (Table 1).

### 2.2 The extent of DBI change in *A. thaliana* roots was larger than that in leaves

Under $K^+$-sufficient condition, the DBI of total lipids in *A. thaliana* root was 3.45 (Table 2); this was much lower than that in leaf, which was 5.35. In addition, the DBI of all lipid classes in *A. thaliana* roots were lower than those in its leaves. This phenomenon also occurred under $K^+$-deficient

conditions, which indicated that the membrane fluidity in *A. thaliana* leaves was superior to that in roots. Under control conditions, the DBI of MGDG was 5.69, which was much higher than those of other lipids. The lowest DBI was exhibited for PG, which was only 2.11. However, in contrast to the case for leaves, there were no clear trends between $K^+$-deficiency stress and changes in DBI in roots. Instead, complex and varied patterns were displayed, and the changes were larger than those in leaves. The DBI of total lipids in *A. thaliana* roots showed a 1.16% decrease, and 0.29% and 4.06% increases under conditions of 0.51, 0.051, and 0 mmol · $L^{-1}$ $K^+$ (Table 2), respectively, compared with the control. However, there was a 0.75% decrease, a 0.56% increase, and a 0.19% decrease in *A. thaliana* leaves under the same $K^+$ conditions (Table 1). The changes of DBI in extraplastidic lipids were larger than those in plastidic lipids.

### 2.3 The patterns of DBI change in *C. himalaica* was similar to that in *A. thaliana*

In *C. himalaica* leaves, the DBI of MGDG was 5.70, which was much higher than those of other lipids. PG had the lowest DBI, which was only 2.41 (Table 1). Under control condition, the DBI of each lipid class and total lipids in *C. himalaica* leaves were lower than those of *A. thaliana*, a phenomenon that also occurred in $K^+$-deficient *C. himalaica* and *A. thaliana* leaves. This indicates that the membrane fluidity in *C. himalaica* leaves was lower than that in *A. thaliana*. Under $K^+$-deficient conditions, changes in the DBI of total lipids in *C. himalaica* leaves showed no significant difference compared with the control. The DBI of extraplastidic lipids showed an increase after the imposition of $K^+$-deficiency. For plastidic lipids, the DBI of DGDG and PG increased after exposure to both 0.051 and 0 mmol · $L^{-1}$ $K^+$. Meanwhile, the DBI of MGDG showed a 0.35%

Table 1 DBI of membrane lipids in *A. thaliana* and *C. himalaica* leaves following exposure to different levels of $K^+$. Values in the same row with different letters differ significantly at $P<0.05$ (Fisher's least significant difference). An asterisk indicates that the value differs significantly from that of *A. thaliana* under the same conditions ($P<0.05$)

| Lipid class | Plant species | Double-bond index | | | | Relative change/% | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | 0.51 /mmol·$L^{-1}$ | 0.051 /mmol·$L^{-1}$ | 0 /mmol·$L^{-1}$ | 0.51 /mmol·$L^{-1}$ | 0.051 /mmol·$L^{-1}$ | 0 /mmol·$L^{-1}$ |
| DGDG | *A. thaliana* | 5.29±0.03$^{a}$ | 5.22±0.05$^{b}$ | 5.22±0.04$^{b}$ | 5.27±0.07$^{ab}$ | −1.32 | −1.32 | −0.38 |
| | *C. himalaica* | 5.20±0.03$^{a*}$ | 5.20±0.02$^{a}$ | 5.16±0.04$^{a*}$ | 5.17±0.01$^{a*}$ | 0 | −0.77 | −0.58 |
| MGDG | *A. thaliana* | 5.88±0.01$^{a}$ | 5.86±0.02$^{ab}$ | 5.86±0.00$^{ab}$ | 5.86±0.01$^{b}$ | −0.34 | −0.34 | −0.34 |
| | *C. himalaica* | 5.70±0.02$^{ab*}$ | 5.72±0.01$^{a*}$ | 5.69±0.02$^{b*}$ | 5.70±0.02$^{ab*}$ | 0.35 | −0.17 | 0 |
| PG | *A. thaliana* | 3.20±0.04$^{a}$ | 3.25±0.14$^{a}$ | 3.26±0.08$^{a}$ | 3.20±0.06$^{a}$ | 1.56 | 1.87 | 0 |
| | *C. himalaica* | 2.41±0.05$^{ab*}$ | 2.43±0.05$^{a*}$ | 2.35±0.02$^{ab*}$ | 2.34±0.02$^{b*}$ | 0.83 | −2.49 | −2.90 |
| PC | *A. thaliana* | 3.92±0.07$^{b}$ | 4.01±0.10$^{a}$ | 4.06±0.04$^{a}$ | 4.06±0.05$^{a}$ | 2.30 | 3.57 | 3.57 |
| | *C. himalaica* | 3.59±0.02$^{c*}$ | 3.79±0.04$^{b*}$ | 3.81±0.05$^{b*}$ | 3.90±0.02$^{a*}$ | 5.57 | 6.13 | 8.63 |
| PE | *A. thaliana* | 3.42±0.04$^{b}$ | 3.41±0.01$^{b}$ | 3.53±0.05$^{a}$ | 3.57±0.03$^{a}$ | −0.29 | 3.22 | 4.39 |
| | *C. himalaica* | 3.27±0.02$^{c*}$ | 3.32±0.04$^{b*}$ | 3.40±0.02$^{a*}$ | 3.44±0.02$^{a*}$ | 1.53 | 3.98 | 5.20 |
| PI | *A. thaliana* | 2.79±0.01$^{b}$ | 2.74±0.04$^{c}$ | 2.80±0.03$^{ab}$ | 2.82±0.04$^{a}$ | −1.79 | 0.36 | 1.07 |
| | *C. himalaica* | 2.59±0.02$^{c*}$ | 2.65±0.01$^{b*}$ | 2.68±0.02$^{ab*}$ | 2.71±0.01$^{a*}$ | 2.32 | 3.47 | 4.63 |
| PS | *A. thaliana* | 2.74±0.03$^{b}$ | 2.71±0.04$^{b}$ | 2.72±0.02$^{b}$ | 2.80±0.03$^{a}$ | −1.09 | −0.73 | 2.19 |
| | *C. himalaica* | 2.58±0.02$^{c*}$ | 2.62±0.02$^{b*}$ | 2.66±0.03$^{a*}$ | 2.69±0.01$^{a*}$ | 1.55 | 3.10 | 4.26 |
| PA | *A. thaliana* | 3.51±0.04$^{b}$ | 3.46±0.11$^{b}$ | 3.56±0.07$^{b}$ | 3.75±0.12$^{a}$ | −1.42 | 1.42 | 6.84 |
| | *C. himalaica* | 3.24±0.05$^{b*}$ | 3.40±0.27$^{ab}$ | 3.23±0.20$^{b*}$ | 3.46±0.13$^{a*}$ | 4.94 | −0.31 | 6.79 |
| Total lipids | *A. thaliana* | 5.35±0.03$^{a}$ | 5.31±0.09$^{a}$ | 5.38±0.03$^{a}$ | 5.34±0.20$^{a}$ | −0.75 | 0.56 | −0.19 |
| | *C. himalaica* | 5.17±0.04$^{a*}$ | 5.25±0.07$^{a}$ | 5.20±0.04$^{a*}$ | 5.20±0.02$^{a*}$ | 1.55 | 0.58 | 0.58 |

Table 2　DBI of membrane lipids in *A. thaliana* and *C. himalaica* roots following exposure to different levels of $K^+$. Values in the same row with different letters differ significantly at $P<0.05$ (Fisher's least significant difference). An asterisk indicates that the value differs significantly from that of *A. thaliana* under the same conditions ($P<0.05$)

| Lipid class | Plant species | Double-bond index | | | | Relative change/% | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | 0.51 /mmol·$L^{-1}$ | 0.051 /mmol·$L^{-1}$ | 0 /mmol·$L^{-1}$ | 0.51 /mmol·$L^{-1}$ | 0.051 /mmol·$L^{-1}$ | 0 /mmol·$L^{-1}$ |
| DGDG | *A. thaliana* | 4.98±0.07$^{b}$ | 5.04±0.11$^{ab}$ | 5.04±0.07$^{ab}$ | 5.17±0.12$^{a}$ | 1.20 | 1.20 | 3.81 |
| | *C. himalaica* | 4.67±0.14$^{a*}$ | 4.69±0.17$^{a*}$ | 4.72±0.09$^{a*}$ | 4.71±0.10$^{a*}$ | 0.43 | 1.07 | 0.86 |
| MGDG | *A. thaliana* | 5.69±0.06$^{a}$ | 5.47±0.44$^{a}$ | 5.68±0.10$^{a}$ | 5.73±0.05$^{a}$ | −3.87 | −0.18 | 0.70 |
| | *C. himalaica* | 5.56±0.04$^{a}$ | 5.37±0.30$^{a}$ | 5.47±0.21$^{a}$ | 5.46±0.25$^{a}$ | −3.42 | −1.62 | −1.80 |
| PG | *A. thaliana* | 2.11±0.04$^{b}$ | 2.20±0.04$^{a}$ | 2.10±0.03$^{b}$ | 2.18±0.05$^{a}$ | 4.26 | −0.47 | 3.32 |
| | *C. himalaica* | 2.15±0.04$^{ab}$ | 2.14±0.04$^{ab*}$ | 2.12±0.05$^{b}$ | 2.17±0.05$^{a}$ | −0.46 | −1.39 | 0.93 |
| PC | *A. thaliana* | 3.50±0.05$^{ab}$ | 3.43±0.03$^{c}$ | 3.44±0.06$^{bc}$ | 3.52±0.02$^{a}$ | −2.00 | −1.71 | 0.57 |
| | *C. himalaica* | 3.55±0.06$^{b}$ | 3.56±0.03$^{b*}$ | 3.68±0.04$^{a*}$ | 3.70±0.11$^{a*}$ | 0.28 | 3.66 | 4.22 |
| PE | *A. thaliana* | 2.88±0.01$^{c}$ | 3.02±0.03$^{b}$ | 3.07±0.02$^{a}$ | 3.10±0.02$^{a}$ | 4.86 | 6.60 | 7.64 |
| | *C. himalaica* | 3.05±0.03$^{c*}$ | 3.11±0.03$^{b*}$ | 3.14±0.04$^{ab*}$ | 3.17±0.05$^{a*}$ | 1.97 | 2.95 | 3.93 |
| PI | *A. thaliana* | 2.50±0.02$^{a}$ | 2.45±0.02$^{b}$ | 2.46±0.02$^{b}$ | 2.47±0.02$^{ab}$ | −2.00 | −1.60 | −1.20 |
| | *C. himalaica* | 2.54±0.04$^{a}$ | 2.49±0.02$^{b}$ | 2.55±0.02$^{a*}$ | 2.55±0.06$^{a*}$ | −1.97 | 0.39 | 0.39 |
| PS | *A. thaliana* | 2.55±0.02$^{a}$ | 2.50±0.02$^{b}$ | 2.56±0.04$^{a}$ | 2.60±0.03$^{a}$ | −1.96 | 0.39 | 1.96 |
| | *C. himalaica* | 2.66±0.03$^{b*}$ | 2.64±0.03$^{b*}$ | 2.74±0.04$^{a*}$ | 2.73±0.06$^{a*}$ | −0.75 | 3.01 | 2.63 |
| PA | *A. thaliana* | 3.29±0.03$^{a}$ | 3.27±0.06$^{a}$ | 3.32±0.03$^{a}$ | 3.32±0.08$^{a}$ | −0.61 | 0.91 | 0.91 |
| | *C. himalaica* | 3.36±0.05$^{a}$ | 3.37±0.05$^{a*}$ | 3.41±0.05$^{a*}$ | 3.39±0.07$^{a}$ | 0.30 | 1.49 | 0.89 |
| Total lipids | *A. thaliana* | 3.45±0.04$^{b}$ | 3.41±0.13$^{b}$ | 3.46±0.04$^{b}$ | 3.59±0.03$^{a}$ | −1.16 | 0.29 | 4.06 |
| | *C. himalaica* | 3.49±0.03$^{bc}$ | 3.44±0.06$^{b}$ | 3.61±0.09$^{a*}$ | 3.58±0.14$^{ab}$ | −1.43 | 3.44 | 2.58 |

increase and a 0.17% decrease under conditions of 0.51 and 0.051 mmol·$L^{-1}$ $K^+$, respectively, compared with the control. The changes of DBI in extraplastidic lipids were larger than those in plastidic lipids. The DBI of PC showed the largest changes, namely, 5.57%, 6.13% and 8.63% increases after exposure to 0.51, 0.051 and 0 mmol·$L^{-1}$ $K^+$, respectively, compared with the control.

Under $K^+$-sufficient condition, the DBI of total lipids in *C. himalaica* root was 3.49 (Table 2), which was much lower than that in leaves, at 5.17. Furthermore, the DBI of almost all lipid classes in *C. himalaica* roots were lower than those in *C. himalaica* leaves, except for PS and PA. This phenomenon was also observed in plants subjected to $K^+$ deficiency, which indicates that the membrane fluidity in *C. himalaica* leaves was superior to that in its roots. This was similar to the case for *A. thaliana*. In *C. himalaica* roots, lower DBI in PG and PI were observed under both $K^+$-sufficient and $K^+$-deficient conditions, whereas the DBI of DGDG and MGDG were much higher than those of other lipids (Table 2). In contrast to leaves, the DBI of most lipid classes and total lipids in roots were higher in *C. himalaica* than in *A. thaliana*, with the exceptions of DGDG and MGDG. This suggests that membrane fluidity in *C. himalaica* roots was higher than that in *A. thaliana*. Under $K^+$-limited conditions, the changes in the DBI of extraplastidic lipids were larger than those of plastidic lipids, and the DBI of PC and PE showed greater changes than those of other lipids.

### 2.4　Total lysoPLs increased in $K^+$-stressed *A. thaliana* and *C. himalaica* leaves

LysoPLs are minor phospholipids of *A. thaliana*, but they are very sensitive to stresses such as extreme temperature and drought. Under $K^+$-deficient conditions, no tendency for change in each lysoPL class in the leaves and roots of *C. himalaica* and

*A. thaliana* was observed (Table 3). We further calculated the total amount of lysoPLs and found that, under $K^+$-deficient conditions, it increased markedly in *C. himalaica* and *A. thaliana* leaves (Table 4). *A. thaliana* leaves showed 17.98%, 29.21%, and 60.67% increases under conditions of mild $K^+$ limitation, severe $K^+$ limitation, and $K^+$ deprivation, respectively, compared with the control. In *C. himalaica* leaves, when compared with the control, total lysoPLs showed 30.00% and 31.11% increases under conditions of 0.051 and 0 mmol · $L^{-1}$ $K^+$. In contrast, no significant changes in this variable were observed in *A. thaliana* and *C. himalaica* roots.

The levels of total lysoPLs in $K^+$-stressed *A. thaliana* leaves and roots were higher than those in *C. himalaica*. At 4 ℃, lysoPC, lysoPE, and lysoPG in *A. thaliana* leaves were 0.09, 0.06, and 0.07 nmol · $mg^{-1}$, whereas when subjected to freezing stress (−8 ℃), they were 0.71, 0.8, and 0.12 nmol · $mg^{-1}$, respectively (Li *et al.*, 2008). This indicated that the level of lysoPL was positively related to the intensity of stress. The higher levels of total lysoPLs in *A. thaliana* under $K^+$-deficient conditions suggest that this species is far more sensitive to $K^+$ deficiency than *C. himalaica*.

### 2.5 The membrane stability of *C. himalaica* was superior to that of *A. thaliana* under $K^+$-deficient conditions

The ratios of DGDG/MGDG and PC/PE are thought to be positively related to membrane stability. Therefore, in this study, we used these ratios as indicators of membrane stability in $K^+$-stressed *A. thaliana* and *C. himalaica*. The ratio of DGDG to MGDG can be used to estimate membrane stability in leaves because they are the major constituents of plastidic membranes in leaves. In the case of PC and PE, these are extraplastidic lipids, which account for considerable proportions of root membrane lipids. Thus, the stability of root membranes and extraplastidic membranes in leaves can be estimated using the PC/PE ratio. $K^+$ deficiency led to a slight decrease in the DGDG/MGDG ratio in *A. thaliana* and *C. himalaica* leaves (Fig. 1A), but this ratio showed increases in $K^+$-stressed *A. thaliana* and *C. himalaica* roots. DGDG/MGDG ratios in *C. himalaica* leaves were significantly higher than in *A. thaliana* under both control and $K^+$-deficient conditions. Furthermore, *C. himalaica* roots also had a higher ratio of DGDG/MGDG than those of *A. thaliana* under the four different $K^+$ conditions.

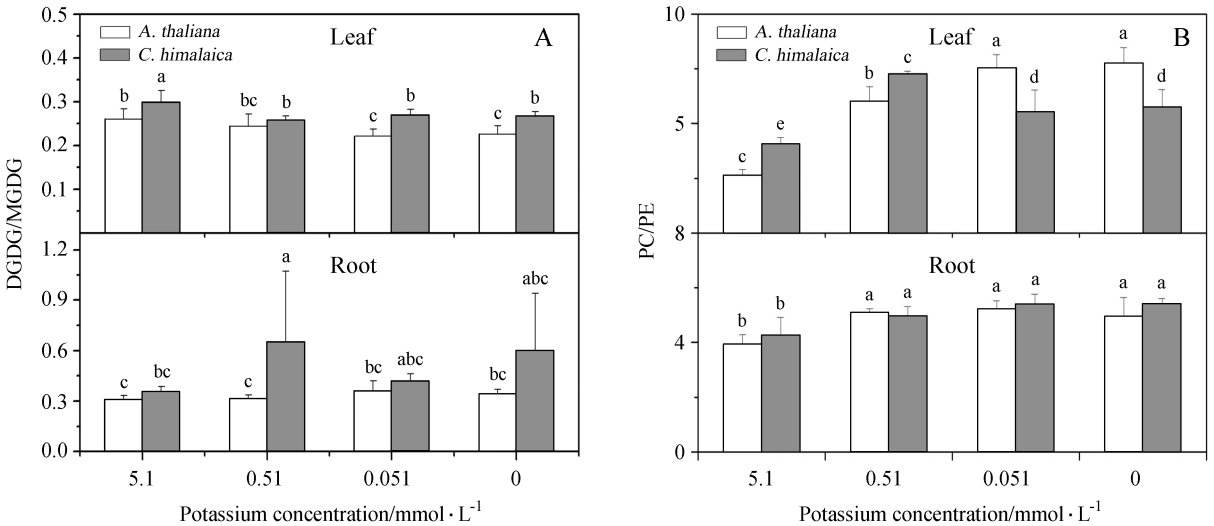


Fig. 1 Changes in DGDG/MGDG and PC/PE ratios in *A. thaliana* and *C. himalaica* under $K^+$-deficient conditions. Data are means ± *SD* ($n$=4 or 5). Bars with different letters differ significantly at $P<0.05$ (Fisher's least significant difference). Blank bars, *A. thaliana*; dark gray bars, *C. himalaica*. A. Changes in DGDG/MGDG following exposure to different $K^+$ conditions; B. Changes in PC/PE following exposure to different $K^+$ conditions

Table 3 Total amount of each class of lysoPLs in *A. thaliana* and *C. himalaica* following exposure to different levels of $K^+$. Values in the same row with different letters differ significantly at $P<0.05$ (Fisher's least significant difference). An asterisk indicates that the value differs significantly from that of *A. thaliana* under the same conditions ($P<0.05$)

| Lipid class | Plant species | Lipid (nmol/mg dry weight) | | | | Relative change/% | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | 0.51 /mmol·L$^{-1}$ | 0.051 /mmol·L$^{-1}$ | 0 /mmol·L$^{-1}$ | 0.51 /mmol·L$^{-1}$ | 0.051 /mmol·L$^{-1}$ | 0 /mmol·L$^{-1}$ |
| LysoPG of leaf | *A. thaliana* | 0.0193±0.0114$^{b}$ | 0.0155±0.0118$^{b}$ | 0.0461±0.0268$^{a}$ | 0.0163±0.0125$^{b}$ | -19.69 | 138.86 | -15.54 |
| | *C. himalaica* | 0.0087±0.004$^{a}$ | 0.0041±0.0016$^{a}$ | 0.0196±0.0079$^{a*}$ | 0.0126±0.0077$^{a}$ | -52.87 | 125.29 | 44.83 |
| LysoPG of root | *A. thaliana* | 0.0047±0.0035$^{ab}$ | 0.002±0.0028$^{b}$ | 0.0055±0.0057$^{ab}$ | 0.0139±0.0071$^{a}$ | -57.45 | 17.02 | 195.74 |
| | *C. himalaica* | 0.0118±0.0133$^{a}$ | 0.0106±0.0072$^{a}$ | 0.0101±0.0084$^{a}$ | 0.0038±0.0042$^{a*}$ | -10.17 | -14.41 | -67.8 |
| LysoPC of leaf | *A. thaliana* | 0.0314±0.0022$^{a}$ | 0.0406±0.0053$^{a}$ | 0.0331±0.0034$^{a}$ | 0.0399±0.0148$^{a}$ | 29.3 | 5.41 | 27.07 |
| | *C. himalaica* | 0.0343±0.0044$^{b}$ | 0.0164±0.003$^{c*}$ | 0.046±0.0055$^{a*}$ | 0.0519±0.0137$^{a*}$ | -52.19 | 34.11 | 51.31 |
| LysoPC of root | *A. thaliana* | 0.189±0.0532$^{b}$ | 0.21±0.0677$^{b}$ | 0.321±0.0565$^{a}$ | 0.209±0.0194$^{b}$ | 11.11 | 69.84 | 10.58 |
| | *C. himalaica* | 0.113±0.0385$^{b*}$ | 0.17±0.0265$^{a}$ | 0.126±0.0028$^{ab*}$ | 0.149±0.0216$^{ab*}$ | 50.44 | 11.5 | 31.86 |
| LysoPE of leaf | *A. thaliana* | 0.0388±0.0086$^{b}$ | 0.0492±0.018$^{b}$ | 0.0362±0.0075$^{b}$ | 0.0866±0.0466$^{a}$ | 26.8 | -6.7 | 123.2 |
| | *C. himalaica* | 0.0422±0.0075$^{ab}$ | 0.0164±0.0018$^{b*}$ | 0.0514±0.0193$^{a}$ | 0.0539±0.0159$^{a*}$ | -61.14 | 21.8 | 27.73 |
| LysoPE of root | *A. thaliana* | 0.239±0.0782$^{a}$ | 0.15±0.101$^{b}$ | 0.182±0.0487$^{ab}$ | 0.151±0.0408$^{b}$ | -37.24 | -23.85 | -36.82 |
| | *C. himalaica* | 0.0906±0.057$^{a*}$ | 0.11±0.0731$^{a}$ | 0.0869±0.0107$^{a*}$ | 0.0641±0.0256$^{a*}$ | 21.41 | -4.08 | -29.25 |

Table 4 Total lysoPL in *A. thaliana* and *C. himalaica* following exposure to different levels of $K^+$. Values in the same row with different letters differ significantly at $P<0.05$ (Fisher's least significant difference). An asterisk indicates that the value differs significantly from that of *A. thaliana* under the same conditions ($P<0.05$)

| Lipid | Plant species | Lipid (nmol/mg dry weight) | | | | Relative change to control/% | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | 0.51 /mmol·L$^{-1}$ | 0.051 /mmol·L$^{-1}$ | 0 /mmol·L$^{-1}$ | 0.51 /mmol·L$^{-1}$ | 0.051 /mmol·L$^{-1}$ | 0 /mmol·L$^{-1}$ |
| Total LysoPLs of leaf | *A. thaliana* | 0.089±0.012$^{b}$ | 0.105±0.022$^{ab}$ | 0.115±0.034$^{ab}$ | 0.143±0.066$^{a}$ | 17.98 | 29.21 | 60.67 |
| | *C. himalaica* | 0.09±0.012$^{a}$ | 0.037±0.003$^{b*}$ | 0.117±0.018$^{a}$ | 0.118±0.034$^{a}$ | -58.89 | 30 | 31.11 |
| Total LysoPLs of root | *A. thaliana* | 0.433±0.124$^{ab}$ | 0.365±0.16$^{b}$ | 0.508±0.099$^{a}$ | 0.374±0.057$^{b}$ | -15.7 | 17.32 | -5.9 |
| | *C. himalaica* | 0.215±0.1$^{a*}$ | 0.291±0.09$^{a}$ | 0.221±0.013$^{a*}$ | 0.206±0.021$^{a*}$ | 35.35 | 2.79 | -4.19 |

Under $K^+$-deficient conditions, a tendency for an increase in the PC/PE ratio was observed for both leaves and roots of both *A. thaliana* and *C. himalaica* (Fig. 1B). Under control and 0.51 mmol·$L^{-1}$ $K^+$conditions, the PC/PE ratio in *C. himalaica* leaves was significantly higher than that in *A. thaliana*. However, when grown under conditions of 0.051 and 0 mmol·$L^{-1}$ $K^+$, *A. thaliana* showed a higher ratio of PC/PE than in the presence of 0.51 mmol·$L^{-1}$ $K^+$. Under the 0.51 mmol·$L^{-1}$ $K^+$ condition, the PC/PE ratio in *A. thaliana* root was higher than that in *C. himalaica*, but under 5.1, 0.051 and 0 mmol·$L^{-1}$ $K^+$ conditions, *C. himalaica* had higher PC/PE ratios than *A. thaliana*.

In summary, the obtained results suggest that, under $K^+$-deficient conditions, the DBI of most lipid classes in *A. thaliana* and *C. himalaica* showed no significant change when compared with that of the control. The DBI of extraplastidic lipids showed an increase under $K^+$-deficient conditions, whereas that of plastidic lipids decreased with the decreasing availability of $K^+$. The membrane fluidity in *A. thaliana* and *C. himalaica* leaves was higher than that in roots. *C. himalaica* roots exhibited greater membrane fluidity than *A. thaliana*; in contrast, the membrane fluidity in *A. thaliana* leaves was superior to that in *C. himalaica*. The total amount of lysoPLs was higher in $K^+$-stressed *A. thaliana* than in *C. himalaica*, and the ratios of DGDG/MGDG and PC/PE were higher in *C. himalaica* leaves and roots than in *A. thaliana*. These findings indicate that the membrane stability of *C. himalaica* leaves and roots is superior to that of *A. thaliana* under $K^+$-deficient conditions.

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